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1991 年 5 月 20 日

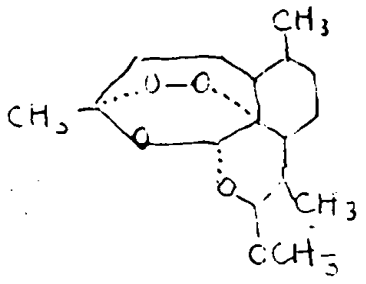
说　　明　　书

抗疟新药—复方蒿甲醚及其制备方法

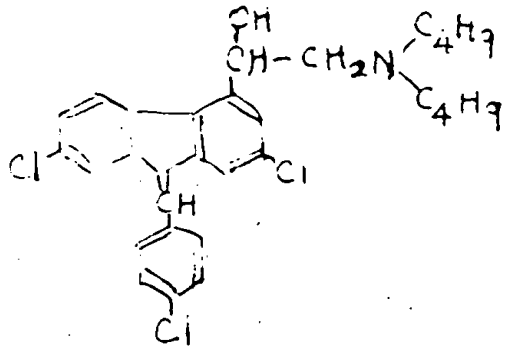
本发明涉及一种抗疟新药复方蒿甲醚及其制备方法。

全世界抗药性疟疾日渐增多，已有70%以上的恶性疟原虫对现有抗疟药如氯喹、氨酚喹、氯胍、乙胺嘧啶和甲氟喹及其复方等，在使用中均产生了不同程度的抗药性。此外，氨酚喹、甲氟喹及其复方等毒副反应也较为严重，直接影响到全世界每年1.03亿疟疾患者和2.64亿疟原虫携带者的治疗。

蒿甲醚（$C_{16}H_{28}O_5$）和本芴醇（$C_{30}H_{32}Cl_3NO$）是近年来研制成功的两种结构和作用不同的新型抗疟药。此两药通常都用单药治疗。蒿甲醚的作用特点是杀疟原虫快速，但杀虫不彻底，治疗后病人血中残留的原虫复燃率高。本芴醇恰恰相反，杀虫较彻底，治愈率高，却奏效缓慢，但是两者的共同点是对抗药性恶性疟疗效显著。



蒿甲醚



本芴醇

本发明的目的是提供一种抗疟新药复方蒿甲醚，是基于运用药物间的增效作用和互补作用原理，通过动物实验研究，确定药物间的作用类型，寻找复方的最佳组成，借以达到扬长避短的目的，既发挥蒿甲醚速效作用的优势，又充分利用本芴醇杀虫彻底，治愈率高的特点。迄至目前，国内外尚无与此同类的抗疟药复方制品。

本发明的目的是通过以下的技术方案来实现的。

一、通过动物试验研究确定蒿甲醚和本芴醇的剂量系列配比关系以及达到最佳配比条件下的增效作用。以伯氏鼠疟原虫（Plasmodium berghei）感染小白鼠为试验模型，采用正交性设计，用“4天抑制试验”法对两药物的不同剂量系列配比进行抗疟效价的平行对比试验，以直线回归方程计算法求出ED_{50}或ED_{90}及其增效指数。

$$\text{增效指数} = \frac{\text{单药}ED_{50}\text{或}ED_{90}}{\text{复方中相应药}ED_{50}\text{或}ED_{90}}$$

按此公式求出本复方抗鼠疟最佳配比为2:0.75（ED_{90}的增效指数＞6）。

在鼠疟实验基础上，进行猴疟原虫（Plasmodium Knowlesi）/恒河猴试验，结果表明复方抗疟的最佳配比为1:3～6。

二、本复方药物间作用类型的评定是按照peters（1969）的相加线图示法测定的，凡是复方ED_{90}的坐标点位于相加线附近者，判为药物间有相加作用，坐标点位于相加线下方，且远离该线者，判为药物间有增效作用，若坐标点位于相加线上方，且远离该线者，判为药物间有拮抗作用，本复方呈药效学增效作用。

三、对复方杀虫速度的判定是用人工感染疟疾的模型动物血中原虫密度增长到高密度时，用大剂量（等效量）即$20\times ED_{90}$/ig给药法，给药后连续观察动物血中原虫的下降速度，按原虫下降90%的时间计算，复方为49.7小时，本芴醇单药为64.3小时，因蒿甲醚未能使原虫密度下降至90%即回升了。可见复方的杀虫速度完全符合设计要求。

四、蒿甲醚和本芴醇组方最佳配比的临床探索。根据动物试验结果，参照蒿甲醚和本芴醇单药临床有效剂量推算西药的最佳配比为1:4～1:6，因此按1:4计量，每片含蒿甲醚25mg，本芴醇100mg，按1:5计量，每片含蒿甲醚20mg，本芴醇100mg以及按1:6计量每片含蒿甲醚20mg，本芴醇120mg，三种配比，选1:5及1:6设两个组，进行临床平行对比试验，两组均用3天4次疗法，即首次口服4片，间隔8、24、48小时各服4片，成人总量16片。选择恶性疟疾患者40例，随机分为两组，试验结果显示两者药后4小时，原虫下降率分别为96.3%和94.2%；平均原虫消失时间为34.8小时和36.0小时，平均退热时间为23.2小时和22.4小时。但28天原虫复燃率1:5组为20%，1:6组则无一复燃，全部治愈。证明在该试验中复方中的蒿甲醚和本芴醇对人疟的最佳配比为1:6。

五、复方延缓原虫产生抗药性作用的判定。通过小剂量递增连续血传法培育原虫抗药株，以此抗药株对复方蒿甲醚和本芴醇单药分别进行抗药性产生速度的平行对比试验，历时560天，连续培育80代，结果证明两药伍用，具有延缓原虫产生抗药性和降低原虫抗性程度的作用。例如培育20代的疟原虫对药物耐受剂量计算，对本芴醇单药的耐受剂量比其对原剂量的耐受力增加100倍，对蒿甲醚则增加20倍，但是对本复方仅增加2.9倍。按第30代抗性指数（I 90）计算，本芴醇＞410，复方＞19.3，说明此试验方法能较确切地评定本复方有延缓原虫产生抗药性的作用，在实际使用中不易产生抗药性。

六、按药理学方法测定复方在临床上的副作用。以小鼠、大鼠和猫为试验模型，复方两药按1:6配制，总用量为112mg/kg（相当于人用一次剂量的10倍），按等容量灌胃，小鼠、大鼠均为10ml/kg，猫2ml/kg，观察期间，对动物的神经系、心血管系及呼吸系等进行检查。但结果均无任何药理活性变化。

七、按毒理学方法对复方进行安全性评价，其中蒿甲醚和本芴醇之比为1:6配制。急性毒性试验用小白鼠半数致死量（LD_{50}），灌胃给药为4455mg/kg，腹腔注射为1554mg/kg。按化学毒力分级标准，该复方属于低毒级。大白鼠和Bea-

gle狗14天毒性试验，各设大、中、小三个剂量组，每天口服一次，连续14天，观察并检查动物的饮食、体重、血液学、生化等指标以及主要脏器和药物靶器官的病理学检查。结果显示大白鼠的基本安全剂量为448MKD，相当于临床剂量的40倍；狗的安全剂量为556MKD，相当于人用剂量的50倍。在大剂量组，靶器官的肝胃，显有异常变化，但停药后28天检查均恢复正常。说明该复方毒性低，安全范围大，无不可逆性毒性反应。

八、复方和单药对人恶性疟疗效比较。设两个试验组，口服给药，采用 3天 4次疗法，各组恶性疟患者20例，比较观察复方及其相应剂量的蒿甲醚和本芴醇单药三者的疗效。

给药后24小时原虫的下降率：复方为97%，蒿甲醚为95.1%，本芴醇为74.5%；平均原虫消失时间：复方为35.6小时，蒿甲醚为38.7小时，本芴醇为68.4小时；平均退热时间复方为23.8小时，蒿甲醚为19.7小时，本芴醇为40小时，28天治愈率，分别为95%，45%和65%，该实验性治疗方案能比较清晰地说明复方和单药在疗效上的差别，复方优于单药。

九、复方的临床扩大试验及氯喹疗效对比试验，复方蒿甲醚口服给药，3天4次疗法，共收治恶性疟疾400例。临床观察的主要指标：(1)平均原虫消失时间（试验结果为23.2～41.0小时）；(2)平均退热时间（结果为20.4～25.7小时）；(3)给药后28天治愈率（结果平均为95～100%），表明复方疗效比蒿甲醚单药（45%）提高1.3倍以上。

复方与氯喹疗效对比试验。复方按3天4次疗法给药，氯喹采用国际标准给药，即首次4片，8、24和49小时各服2片，成人总量10片。复方组患者35例，氯喹组患者22例。观察内容：(1)平均原虫消失时间（结果分别为37.8和87.3小时）；(2)平均退热时间（结果分别为24.2和58.5小时）；(3)28天治愈率（结果分别为97.1%和40.9%）。证明复方对于抗氯喹疟疾有显著疗效，明显优于氯喹1.4倍以上。

对患者血、尿及心电图检查均无异常发现。

根据本发明复方蒿甲醚提出的技术方案给出的实施例如下：

实施例 1：通过动物试验研究，确定复方中蒿甲醚和本芴醇的剂量系列关系，以及两药联合后的增效作用。以伯氏疟疾原虫（Plasmodium berghei）感染的小白鼠为试验模型，采用正交性设计，“4天抑制试验”法对两药的不同剂量系列配比进行抗疟效价平行对比试验，以直线回归方程计算法求出ED_{50}或ED_{90}及其增效指数。

$$\text{增效指数} = \frac{\text{单药}ED_{50}\text{或}ED_{90}}{\text{复方中相应药}ED_{50}\text{或}ED_{90}}$$

按此公式求出复方抗鼠疟的最佳配比。

在鼠疟试验基础上，以诺氏猴疟原虫（Plasmodium Knowlesi）/恒河猴为模型进行试验。每只猴静脉接种5×10 寄生疟原虫的红细胞。当动物血液中原

虫寄生率达3～5%时，按设计方案开始给药（灌胃）。首次给药后每12小时涂片镜检一次，原虫转阴后改为每日一次，15天后隔日一次，连续观测105天，根据药效学标准判定试验结果。

实施例 2：本复方药物间作用类型的评定是按照Peters（1969）的相加线图示法测定，凡是复方ED 的坐标点位于相加线附近者，判为药物间有相加作用，坐标点位于相加线下方，且远离该线者，判为药物间有增效作用，若坐标点位于相加线上方，且远离该线者，判为药物间有拮抗作用。本复方呈药效学增效作用。

实施例 3：复方杀虫速度的判定。用人工感染疟疾的模型动物血中原虫密度增长到高密度时，用大剂量（等效量）即$20\times ED_{90}$/ig给药法，给药后连续观察动物血中原虫的下降速度，按原虫下降90%的时间计算其下降的时间、速度所得出的数据为复方的杀虫速度。

实施例 4：蒿甲醚和本芴醇组方最佳配比的临床探索。根据动物试验结果，参照蒿甲醚和本芴醇单药的临床有效剂量推算两药的最适配比为1:4、1:5和1:6（例如按1:6计算蒿甲醚为20mg，本芴醇为120mg），按1:5和1:6两个配比分组，进行临床平行对比试验，均用3天4次疗法，即首次口服4片，间隔8、24及48小时各服4片，成人总量16片。选择恶性疟疾患者40例，随机分为两组 。要求此项试验结果能揭示两组给药后：(1)24小时原虫最大下降率；(2)平行原虫最短消失时间和(3)平行最快退热时间，以此阐明复方最佳剂量配比的临床疗效。

实施例 5：复方延缓原虫产生抗药性作用的判定。用小剂量递增连续血传培育疟原虫抗药株，以此抗药株对复方和蒿甲醚、本芴醇单药分别进行抗药性产生速度的平行对比试验，连续培育80代，历时 560天，比较观察疟原虫对复方和单药的耐受性，其耐受性越大，抗药性越强，例如培育20代的疟原虫对药物耐受剂量计算结果，对本芴醇单药的耐受力比其对原剂量的耐受力增加100倍，但对复方的耐受力仅增加 2.9倍。此项试验能比较确切地说明复方有延缓原虫产生抗药性的作用。

实施例 6：按药理学方法测定复方在临床上的副作用，以小鼠、大鼠和猫为试验模型，复方中的两药按1:6配制，总用量为112mg/kg，（相当于人用一次剂量的10倍）。按等容量灌胃，小鼠和大鼠为10ml/kg，猫2ml/kg，观察期间，对动物的神经系、心血管及呼吸系进行检查，但均无药理学活性变化。

实施例 7：按毒理学方法对复方进行安全性评价，其中蒿甲醚和本芴醇之比为1:6配制。急性毒性试验用的小白鼠半数致死量（LD50）灌胃给药为 4455 mg/kg，腹腔注射为1554mg/kg。按化学毒力分级标准，该复方属于低毒级。大白鼠和Beagle狗14天毒性试验，各设大、中、小三个剂量，每天口服一次，连续14天，观察并检查动物及饮食、体重、血液学、生化等指标以及主要脏器和药物的靶器官的病理学检查。结果显示，大白鼠的基本安全剂量为448MKD，相当于临床剂量的40倍，狗的安全剂量为556MKD，相当于人用剂量的50倍。但均无不可逆性毒性反应。

实施例 8：复方和单药对人恶性疟疾的疗效比较。设两个试验组，口服给药，采用3天4次疗法；各组恶性疟疾患者20例，分别比较观察复方及其相应剂量的蒿甲醚和本芴醇单药三者的疗效，包括：(1)给药24小时的原虫下降率；(2)平行原虫消失时间和(3)28天治愈率。此试验性治疗方案能进一步指出复方与单药在疗效上的差别。

实施例 9：复方的临床扩大试验及与氯喹疗效对比试验。采用3天4次疗法，口服给药。共收治恶性疟疾400例，主要观察指标：(1)平均原虫消失时间（结果为23.2～41.0小时）；(2)平均退热时间（结果为20.4～25.7小时）；(3)28天治愈率（结果平均治愈率为96.8%）。

复方与氯喹疗效对比试验。复方以3天4次疗法给药，氯喹采用国际标准法给药，即首次4片，8、24和48小时各服2片，成人总量10片，复方设患者35例，氯喹为22例，观察内容为：(1)平均原虫消失时间（结果为37.8和87.3小时）；(2)平均退热时间（结果97.1%和40.9%）。证明复方对于抗氯喹疟疾治疗有显著效果。

实施例10：为了适应不同情况的患者服用需要，本复方除制成口服片剂外，同样可制成其他剂型如胶囊、胶丸、栓剂、脂质体和透皮剂型等。

实施里11：

(1)实验动物：

㈠雄性瑞士种昆明远交品系小白鼠，体重20（±2）克；

㈡恒河猴，雌雄均可，体重2～3kg。

(2)实验疟原虫：

伯氏疟原虫敏感株（P. berghei K.173）；诺氏疟原虫（P.Konwlesi），人恶性疟原虫（P.faleiparum）（得自中国海南岛抗氯喹恶性疟流行区）。

(3)鼠疟"4天抑制试验）和"4天治疗试验"法是取感染 5天的供血小白鼠含疟原虫血液，肝素抗凝，生理盐水稀释至每0.2ml含10 个寄生疟原虫的红血球，每只小白鼠以0.2ml/腹腔接种。接种日为D 、次日为D ，依次类推。两者的区别是前者感染当天即开始给药，后者是感染后阳性反应鼠疟原虫密度达 5～15%时开始给药。猴疟105天观察法是每只猴静脉接种5×10 个寄生疟原虫的红血球、待原虫寄生率达5%左右开始灌胃给药，每天1次，连续7天，首次给药后每12小时涂血片查疟原虫至原虫转阴后，每日1次，2周后每 3天1次，至105天为止。

(4)药物及其配制：蒿甲醚和本芴醇于实验前两天按设计剂量加吐温（Tween30），研磨成水乳状悬液，小白鼠灌胃溶剂为每次每只0.2ml，猴灌胃每次为1ml/kg。

US

VERIFICATION OF A TRANSLATION

I, the below named translator, hereby declare that:

My name and post office address are as stated below:

That I am knowledgeable in the English language and in the language in which the below identified international application will be filed, and that I believe that the attached English translations of the People's Republic of China patent application 90106722.9 is true and complete translations of the above-identified international application as filed.

Date: July 27, 1991

Full Name of the Translator (typed or printed): Jiashan WU 吴嘉善

Signature of the Translator: Jiashan Wu 吴嘉善

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TITLE

A Novel Antimalarial Drug-Compound Artemether And Its Method of Preparation

The invention is related to a new antimalarial drug compound artemether, and its method of preparation.

The number of drug-resistant malaria cases is increasing day by day throughout the world. The drug-resistance to various degrees has been emerged in more than 70% of the maliguant malarial protozoa during the application of existing antimalarial drugs, such as chloroquine, amodiaquine, chlorquanide, pyrimethamine, mefloquine and their complex prescriptions. Besides, the toxic side-effects of amodisquine, mefloquine and their complex prescriptions are rather serious thus influencing directly the therapeutic outcome in 103 million malarial patient and 264 million parasite-carries each year.

Artemether ($C_{13}H_{28}O_5$) and benflumetol ($C_{32}H_{32}Cl_3NO$) are two new-types of antimalarial drugs with different structure and function which were successfully developed in recent years. Usually they are used singly. Artemether is characterized by rapid but incomplete killing of parasites with high recrudescent rate of the survived protozoa in the blood of patients after treatment.

On the contrary, benflumetol kills the protozoa completely and the cure rate is high, but its therapeutic effect appears slowly. The common feature of these two drugs is their remarkable curative effect against drug-resistant malignant.

The purpose of the present invention is to provide a new anti-malarial drug, compound artemether, to put into use of the synergism and complementation between drugs, to determine by animal experiments the mode of interaction of drugs, and to find out the optimal ratio of combination between artemether and benflumetol, so that the strong points of each component drug can be brought into full play and their shortcomings be overcome. There is no similar antimalarial complex prescription of this kind up to now appeared both at home and abroad.

The purposes of the present invention have been realized by the following technical scheme.

I. Investigation of serial dose ratios of combination between artemether and benflumetol through animal experiments in order to achieve synergism under optimal ratio of combination. Mice infected with plasmodium berghei were used as the test model. Using orthogonal design, parallal contract experiments were carried out for different doses of these two drugs with "4-day-inhibition test" method, ED50 or ED90 and the synergistic indices were then calculated by means of a linear regression equation.

Plasmodium Knowlesi tests on rhesus wee performed based on the results of the experiments using Plasmodium berghei, and the result showed that the optimal ratio of artemether and

benflumetol in this complex prescription against malaria is 1:3-6.

II. Evaluation of the mode of action between the component drugs of this complex prescription was performed by the method of addition line after Peters (1968, in Am Trop Med Parasital, 62:488-494) . All of the coordinate points of ED90 of a complex prescription located near the addition line were interpreted as additive, the points below and far from the addition line as synergistic, while the point at the upper part of that line and far from it as antagonistic interaction between drugs. As the result, the present complex prescriptoin revealed a synergism in currative effect.

III. The speed of killing protozoa was determined using animal models with artificially infected malaria. When the protozoa in the blood of animals increased to a high density, lare dose (equivalent effective dose) , ie. 20XED90/ig was given. The speed of decrease of protozoa in blood was observed uninterruptedly after administration. The times requied for 90% decrease of the protozoa was 49. 7 hours for the complex prescription and 64. 3 hours for benflumetol alone. Artemether alone could not kill protozoa to as much as 90% before their number inceased again . It is evident that the speed of killing protozoa of this complex prescription meets well the designed requirement.

IV. Clinical exploration of the optimal ratio of combination between artemether and benflumetol. According to the results of animal experiments with reference to the respective clinical effective doses of arte and benf., the optimal ratio of

combination of these two drugs was calculated to be 1:4-1:6. Thus, when 1:4 was taken into account, the doses of arte. and benf. in each tablet were 25mg and 100 mg, respectively. The doses of arte. and benf. would be 20mg and 100mg when the ratio 1:5 is used and be 20mg and 120mg respectively for 1:6. Two groups with 1:5 and 1:6 ratios were set up for clinical parallel comparison trials, in which the "3 days and 4 doses" scheme was adopted, i. e. 4 tablets were administeted at the first time and then 4 tablets each for three more times with 8, 24 and 48 hour intervals. That made altogether 16 tablets for each adult. 40 cases of permicious malaria were selected and divided randomly into two groups. The results showed that at 4 hours after administration the ratio of decrease of protozoa in these two group were 96. 3% and 94. 2%, the time for disappearance of protozoa were 34. 8hr and 36. 0hr, and the average times for subsidance of fever were 23. 2hr and 22. 4hr, respectively. However, the recridescance rate on 28th day in 1:5 group was 20%, while 0% in 1:6 group (i. e. all of the patients in this group were cured). There results indicate that the optimal ratio of combination of artemether and benflumetol in the complex prescription for treatment of human malaria is 1:6.

V. Determination of the effect of the complex prescription on delaying the formation of drug-resistance by the protozoa. Drug-resistant strains of protozoa were cultivated by the method of increment of small dosoge in serial blood passages. Parallel comparison experiments to evaluate the speed of formation of drug-resistance of these strains given compound artemether and

benflumetol alone were peformed separately in 560 days with serial cultivation for 80 generations. The results showed that artemether and benflumetol, when used in combination, could delay the formation of drug-resistance by protozoa and decrease their magnitude of resistance. For example, the dose of drug tolerated by the 20th generation of cultivated protozoa was 100 folds of the original dose for benf. alone and 20 folds for artemethor alone, but only 2.9 folds for compound arte . As for the index of drug-resistance calculated based on that of the 30th generation (I90), benflumetol > 410 and compound artemether >19.3. It is evident that this experimental method can evaluate more accurately that the compound artemether has the action of delaying the formation of drug-resistance by the protozoa. Thus, the drug-resistance is not liable to be formed in practical use of compound artemether.

VI. Pharmarcological evaluation of the clinical side-effect of the compound artemether. Experiments were carried out in mice, rats and cats models and the complex prescription was prepared with the ratio of combination of two drugs by 1:6. The total dose was 112 mg/kg (equivalent to 10 folds of one dose for humans). Drugs were introduced into stomach with equal volume, i.e. 10ml/kg for mice and rats and 2ml/kg for cats. Neurological, cardiovascular and respiratory systems were examined during the observation period. The result showed that no pharmacological side-effects were found.

VII. Pharmacotoxicological evaluation of the safety of the compound artemether. The ratio of combination of 1:6 for artemether and benflumetol was used in these experiments. The

median lethal dose (LD50) for albino mice was found in acute toxicity experiments to be 4455mg/kg for intraperitoneal injection. Based on grading criteria for chemical toxicity, this complex prescription is of low grade of toxicity. Toxicity experiments for 14 days were performed in rats and beagles, which were divided into high-, medium-and low-dose groups. Drugs were administered per as once every day for successive 14 days. Appetite and body weight were observed, hematological and biological parameters were determined, and pathological examination were made in major viscera and target organs of the drugs. The results revealed that the basic safety dose in rats was 448 MKD, being equivalent to 40 folds of the dose used clinically and 556 MKD in days, equivalent to 50 folds of the dose used in humans. Although abnormal changes were found in target organs (liver and kidney), they recovered to normal on day 28 after last dose. These results indicated that the toxicity of the complex prescription is low, and the safety range is wide and from irreversible toxic reaction.

VIII. Comparision of curative effect of the compound and single drugs for human pernicious malaria.

Two test groups were set up with the 3 days and 4 doses treatment scheme for comparison of the curative effects of compound artemether, artemether and benflumetol . Each group consisted of 20 patients with pernicious malaria.

The rates of decrease of protozoa at 24 hours after administration were found to be 97%, 95. 1% and 74,. 5% for compound artemether, artemether and benflumetol respectively. The

time for disappearance of protozoa were 35.6hr, 38.7hr and 68.4hr, the average time for subsidence of fever were 23.8hr, 19.7hr and 40hr. and 28-day cure rates were 95%, 45% and 65%, respectively. This experimental therapeutic scheme indicated clearly the difference in curative effect between compound and single drugs, i.e. the former is better than the latter two.

IX. Extended clinical trial of compound artemether and comparison of the curative effect with chloroquine.

Four-hundred patients with pernicious malaria were treated with compound artemether, which is administered per or with the 3 days and 4 doses scheme. The main clinical parameters determined and the results were: (1) average time of disappearance of the protozoa (the results were 23.2-41.0hr); (2) average time for subsidence of fever (20.4-25.7hr); (3) cure rate on 28th day after administration (averaged 95-100%). Thus, the curative effect of compound artemether increased more than 1.3 folds than that of artemether alone (45%).

In trails for comparison between the curative effects of compound artemether and chloroquine, the former was administered with 3 days and 4 doses scheme and the latter with the international standard, i.e. 4 tablets in first dose and then 2 tablets each at 8, 24 and 49 hr. with a total of 10 tablet for adults. 35 patients were treated with compound artemether and 22 with chloroquine. The follwing parameters wee determined : (1) average time for disappearance of protozoa (the results were 37.8 hr for compound artemether and 87.3 hr for chloroquine) ; (2) average time for subsidence of fever (24.2hr vs 56.5hr); (3) cure rates on 28th day (97.1% vs 40.9%). these results showed

that the curative effect of compound artemether against plasmodium berghei is evident and is remarkably more than 1.4 folds better than that of chloroquine.

No abnormalities were found in blood, urine and ECG examinations in these patients.

The embodiment examples of the technical schemes proposed in this invention for putting the compound artemether into effect are as follows:

Example 1: Determination of the serial dose relationship between artemether and benflumetol in complex prescription through animal experiment and of the synergism when these two drugs are mixed together. The experimental model used was albino mouse infected with plasmodium berghei. Using orthogonal design and the "4 days inhibition test", parallel experiments were carried out to compare the antimalarial effect of different dose ratios of these two drugs and their ED50 or ED90 and the index of synegism were calculated by means of the linear regression equation.

$$\text{Index of synergism} = \frac{\text{ED50 or ED90 for single drug}}{\text{ED50 or ED90 for that drug in complex prescription}}$$

The optimal ratio of drugs for antimurine malarial effect of the complex prescription was calculated with this equation.

On the basis of experiments in murine malaria, experiments in rhesus monkey model were performed. Each monkey was inoculated intravenously with 5x10 red blood cells parasitized with plasmodium Knowlesi. When the parasitizing rate by the

protozoa amounted to 3-5%, drugs were introduced into the stomach according to designed scheme. Blood smears wee prepared once every 12 hours after the first dose, once everyday after disappearance of protozoa, and once every other day from day 15 onwards till day 105. The experimental results were evaluated according to pharmacodynamical criteria.

Example 2: The mode of interaction between drugs in this complex prescription was determined by figurative method of addition line after Peters (1969). All the coordinate points of ED90 of the complex prescription located near the addition line were interpreted as additive, points located below that line and far from it as synergistic, and points located at the upper part of that line and far from it as antagonistic interaction between drugs. This complex prescription showed pharmacodynamic synergistic action

Example 3: Determination of the speed of killing protozoa. When the protozoa in blood of animals infected artificially with malaria increased to a high density, large dose (equivalent effective dose) of drug (20xED90/ig) was given and the speed of decrease of protozoa in animal blood was observed uninterrupledly after administration . According to the data of time and speed required for 90% decrease of the protozoa, the speed of killing protozoa effected by the complex prescription was thus determined.

Example 4. Clinical evaluation of the optimal ratio of dose combination between artemether and benflumetol in the complex prescription. Based on the result of animal experiment with reference to the clinical effective doses of artemether and

benflumetol singly, the optimal ratio of dose combination of these two drugs was calculated to be 1:4, 1:5 and 1:6. For example, when 1:6 was taken into account, the doses of artemether and benflumetol in each tablet would be 20mg and 120mg, respectively). Two groups of patients given the complex prescription with 1:5 and 1:6 ratios were set up for clinical parallel comparison trials. In both groups the "3 days and 4 doses" treatment scheme was adopted, i. e. 4 tablets were administered at the first time and then 4 tablets each for three more times with 8, 24 and 48 hour intervals. That made altogether 16 tablets for each adult. 40 cases of pernicious malaria were selected and divided randomly into two groups. The following parameters were required to reveal in these two groups after administration : (1) maximal rate for decrease of protozoa at 24 hours; (2) averaged shortest time for disappearance of protozoa; and averaged quickest time for subsidence of fever. By doing so, the clinical curative effect of the complex prescription with optimal ratio of dose combination could be elucidated.

Example 5. Determination of the effect of the complex prescription on delaying the formalion of drug-resistance by the protozoa. Drug-resistant strains of protozoa were cultivated by the method of increment of small dosage in serial blood passages and these strains were used for the determination.

The tolerance of protozoa to the complex prescription and artemether and benflumetol single were observed comparatialy and found that the higher their tolerance, the stronger their

resistance. For example, the tolerant dose by the protozoa of 20th generation to drugs were calculated to be 100 folds of the original tolerable dose of benflumetol while only 2.9 folds of the original dose of compound artemether . These experiment could indicate clearly that the complex prescription has the effect of delaying the formation of drug-resistance by protozoa.

Example 6. Pharmacological determination of the clinical side-effect of the complex prescription. Using mice, rats and cats as experimental models with the 1:6 ratio of dose combination of two drugs. The total dose used was 112mg/kg, being equivalent to 10 folds of the dose administered once in humans. The drug was introduced into stomach with equal volume, i.e. 10ml/kg for mice and rats and 2ml/kg for cats. Neurologcal, cardiovascular and respriatory system were examined during the observation period and no changes of pharmacological side-effect were found.

Example 7: toxicological evaluation of safety of the complex prescription was carried out with the ratio of combination 1:6 The median lethal dose (LD50) for albino mice was found by acute toxicity experiment to be 4455 mg/kg for stomach administration and 1554 mg/kg for intraperitoneal injection. According to the grading criteria for chemical toxicity, this complex prescription belongs to low grade of toxicity. In the 14-day toxicity experiments in albino rats and beagles, which were divided into high-, medium- and low-dosage groups, drugs were administered per or once a day for successive 14 days. Animals were observed for appetite and body weight, and hematological and biochemical parameters were determined. Pathological

examination of major viscera and target organs of the drug were also made. The results demonstrated that the basic safety dose for albino rats was 448MKD, being equivalent to 40 folds of the clinical dosage, and 556MKD for days, equivalent to 50 folds of the dose used in humans. No irreversible toxic reactions were found.

Example 8. Comparison between complex prescription and singl drugs for curative effect against pernicious malaria. two groups were set up with oral administration and the 3 days and 4 doses treatment scheme. There were 20 patients with pernicious malaria in each group. The curative effect of the complex prescription and artemether and benflumetol singly were compared separately. The doses of these two drug in single use were equivalent to that of the complex prescription. The parameters determined were : (1) the rate for decrease of protozoa at 24 hr post administration; (2) average time for disappearance of protozoa; and (3) cure rate on 28th day. This experimental therapeutic scheme could indicate further the difference in curative effect between the complex prescription and the single drugs.

Example 9: Extended clinical trails for the complex prescription and experiment for comparison of the curative effect between chloroquine and the complex prescription. With the 3 days and 4 doses scheme and oral administration, altogether 400 patients with pernicious malarial were treated. Main parameters observed were: (1) average time for disappearance of protozoa (the results were 23.2-41.0hr); (2) average time for subsidence of fever (20.4-25.7hr); (3) 28-day

cure rate averaged (96.8%).

Comparative experiment for curative effect between the complex prescription and chloroquine. The complex prescription was administered with the 3 days and 4 doses treatment scheme and chloroquine was given with the international standard, i.e. 4 tables at the first time and then 2 tablets each time at 8, 24 and 48 hr with a total of 10 tablets for adults. 35 patients were given with the complex prescription and 22 patients with chloroquine. The parameters observed were : (1) average time for disappearance of protozoa (the results were 37.8 hr vs 87.3 hr); (2) average time for subsidence of fever (24.2 hr vs 56.5hr); (3) 28-day cure rate (97.1% vs 40.9%). These results demonstrated remarkable curative effect of the complex prescription against chloroquine -resistant malaria

Example 10: For suiting the needs of the patients under various conditions, besides tablets for oral administration, this complex prescription can also be made into other dosage forms such as ca psule, pill, suppository, liposome, transcutancous form, and so on.

Example 11:

(1) Experimental animals:

(i) Male outbred Swiss-Kunming strain of albino mice, body weight 20(±2) gm;

(ii) Male or female rhesus monkeys, body weight 2-3 kg;

(2) Experimental malarial protozoa: seusitive strains of Plasmodium berghei K173, Plasmsdium Knowlesi and Plasurodium Falciparum (obtained from the epidemic region of

chloroquine-resistant malaria in Hainau lslnd, China)

(3) Experiments on murine malaria with the 4 days inhibition test and the 4 days treatment test. Blood with malarial protozoa was taken from donor mice infected 5 days ago. Hepasine was used as anticoagulant. Blood samples were diluted with normal saline so as there were 10 red blood cells parasitized with protozoa per 0. 2ml, and every mouse was inoculated intraperitoneally with 0. 2ml of that diluted blood.

CLAIMS

1. A novel antimalarial drug-compound artemether, its characteristics lie in: 1) artemether and benflumetol are prescribed as a complex prescription; 2) The optimal ratio of the two drugs presented in the complex prescription is determined through experiment; 3) for suiting the needs of ptients under various conditions, the complex prescription can be made into different dosage forms.

2. Said compound artemether as in claim 1. its characteristic lies in the utilization of the principle of synergism and complementation and under the condition for determining the optimal ratio of artemether vs. benflumetol based upon the results of experiment, to make a preparation of compound artemether which exhibits a curative effect 1.3 folds higher than that of artemether alone and elicits no drug-resistance.

3. Said compound artemether as in claim 1, its characteristic lies in the determination of the optiomal ratio for antimurin malaria complex prescription which in turn is determined through the "4 days inhibition test" and ED 90 calculated by means of the linear regression equation, the optimal ratio so determined is 2:0.75 (the index of synergism of ED90 76).

4. Said compound artemether as in claim 1, its characteristic lies in the optimal ratio of the complex prescription for anti-moukey malaria is 1:3-6.

5. Said compound artemether as in claim 1, its

characteristic lies in determination of the optimal ratio of the complex prescription for anti-human pernicious malaria through extended clinical trails, the optimal ration so determined is 1:4-7.

6. Said compound artemether as in claim 1, its characteristic lies in its dosage form, an oral tablet form of compound artemether is prepared for the needs of adult patients.

7. Said compound artemether as in claim 1, its characteristic lies in its dosage form, an orl syrup form of compound artemether is prepared for the needs of child patients.

8. Said compound artemether as in claim 1, its characteristic lies in its dosage form, capsule of compound artemether may be preapred for the needs of patients suffering from gastrointestinal diseases.

9. Said compound artemether as in claim 1, its characteristic lies in its dosage form as suppository of compound artemether.

10. Said compound artemether as in claim 1, its characteristic lies in its dosage form, a liposome form of compound artemether may be prepared to meets the needs for patients who require a rapid absorption of the drug.

11. Said compound artemether as in claim 1, its characteristics lies in its dosage form, a topical form of compound rtemether effecting through skin may be prepared for the needs of paraoral administration.

ABSTRACT

The invention relates to a new antimalarial drug-compound artemether and its method of preparation. Said priscription is consist of artemether and benflumetol. The priscription have remarkable synergistic effect against malarial and can dimish the resistance against the artimalarial drug such as chloroquine.